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(71) Applicant: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY OF AGRICULTURE** [US/US]; 1400 Independence Avenue, SW, Washington, DC 20250-0302 (US).

(72) Inventor: **HORN, Carsten**; Laerchenweg 4, D-64559 Nauheim (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **HANDLER, Alfred, M.** [—/—]; 6825 NW 52 Terrace, Gainesville, FL 32653 (US).

(74) Agents: **PENDORF, Stephan A.** et al.; Pendorf & Cutliff, 5111 Memorial Highway, Tampa, FL 33634-7356 (US).

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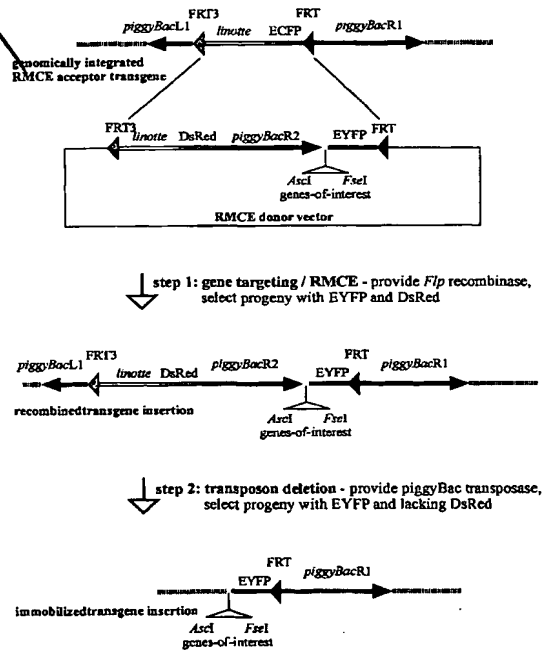
(54) Title: SYSTEMS FOR GENE TARGETING AND PRODUCING STABLE GENOMIC TRANSGEN E INSERTIONS



(57) Abstract: The novel germ-line transformation systems disclosed in this patent application allow the physical deletion of transposon DNA following the transformation process, and the targeting of transgene integrations into predefined target sites. In this way, transposasemediated mobilization of genes-of-interest are excluded mechanistically and random genomic integrations eliminated. In contrast to conventional germ-line transformation technology, our systems provide enhanced stability to the transgene insertion. Furthermore, DNA sequences required for the transgene modification (e.g.transformation marker genes, transposase or recombinase target sites), are largely removed from the genome after the final transgene insertion, thereby eliminating the possibility for instability generated by these processes. The RMCE technology, which is disclosed in this patent application for invertebrate organisms (exemplified in *Drosophila melanogaster)* represents an extremely versatile tool with application potential far beyond the goal of transgene immobilization. RMCE makes possible the *targeted* integration of DNA cassettes into a specific genomic loci that are pre-defined by the integration of the RMCE acceptor plasmid. The loci can be characterized prior to a *targeting* experiment allowing optimal integration sites to be pre-selected for specific applications, and allowing selection of host strains with optimal fitness. In addition, multiple cassette exchange reactions can be performed in a repetitive way where an acceptor cassette can be repetitively exchanged by multiple donor cassettes. In this way several different transgenes can be placed precisely at the same genomic locus, allowing, for the first time, the ability to eliminate genomic positional effects and to comparatively study the biological effects of different transgenes.

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